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MiR-513a-3p inhibits EMT mediated by HOXB7 and promotes sensitivity to cisplatin in ovarian cancer cells

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Abstract. – OBJECTIVE: Our aim was to investigate the biological function and mechanism of action of miR-513a-3p in ovarian cancer cells.

MATERIALS AND METHODS: In this study, qRT-PCR, Western blots, and immunohistochemistry experiments were among the methods used to examine the expression of miR-513a-3p, HOXB7, and related transcripts within ovarian cancer cells. An MTT assay was conducted to evaluate the viability of ovarian cancer cells in the presence of cisplatin. Transwell and wound-healing assays were performed to examine cell migration and invasion. Dual-Luciferase reporter assays were used to evaluate interactions among the aforementioned target genes. *In vivo* tumorigenesis experiments were conducted to verify biological effects of miR-513a-3p and HOXB7.

RESULTS: HOXB7 expression was relatively higher and MiR-513a-3p expression was relatively lower in ovarian cancer cells. Down-regulated expression of miR-513a-3p promoted cell movement *via* its ability to regulate epithelial-mesenchymal transition (EMT). Furthermore, decreased expression of miR-513a-3p resulted in increased sensitivity to cisplatin and resulted in poor prognosis in ovarian cancer patients who had relapsed after treatment with cisplatin. However, HOXB7 reversed the impact of miR-513a-3p in ovarian cancer cells. These results suggested that miR-513a-3p altered EMT mediated by HOXB7 and cisplatin-resistance.

CONCLUSIONS: MiR-513a-3p plays a critical role in promoting sensitivity to cisplatin and tumorigenesis *via* targeting HOXB7 in ovarian cancer.

Key Words:
MiR-513a-3p, HOXB7, Cisplatin, Ovarian cancer.

Introduction

Ovarian cancer is one of the most common malignancies diagnosed in adult women. The incidence of ovarian cancer is increasing globally. Ovarian cancer has the highest mortality among malignancies of the female reproductive tract. Previous studies have shown that ~70% of the patients with ovarian cancer were diagnosed at an advanced stage; the inability to diagnose this malignancy at early stage is most likely related to the lack of effective detection methods[1,2]. Currently, surgical management followed by chemotherapy with a platinum-based drug remains the main treatment for ovarian cancer. Cisplatin is among the drugs used most commonly to treat women with ovarian cancer. However, this regimen can be subverted by drug resistance which limits the efficacy of chemotherapy, which ultimately leads to recurrence, metastasis, and poor prognosis[3,4]. Epithelial-mesenchymal transition (EMT) is a critical factor underlying resistance to chemotherapy[5]. The biological mechanisms that promote and maintain ovarian cancer have not been completely clarified. Given the negative sequelae associated with this disease, it will be critical to identify novel biomarkers for early diagnosis which may lead to more effective treatment for ovarian cancer patients.

MicroRNAs (miRNAs) are a class of 18-25-nucleotide non-coding small RNAs that are expressed widely in somatic organs and tissues. miRNAs can regulate cell migration, invasion, drug resistance among other properties related to carcinogenesis. The relevant miRNAs that have been identified thus far include those that are oncogenes and others that serve molecular targets for early diagnosis and treatment. MiRNAs promote drug resistance and EMT of cancer cells by regulating the expression of specific target genes[6,7]. Of note, miR-200b and miR-15b inhibited the expression of BMI1 and suppressed metas-

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tasis of cisplatin-resistant tongue squamous cell carcinoma by reversing cisplatin-induced EMT[8]. Likewise, miR-3656 mediates EMT by targeted inhibition of RhoF expression and reversed Gissi-4 resistance in a subset of pancreatic cancer patients[9]. Inhibition of miR-200b also induces EMT and resistance to the chemotherapeutic agent, adriamycin[10]. However, the specific effects of miRNAs toward regulating cisplatin-resistance and cisplatin-induced EMT remain unclear.

In this study, we examined expression of miRNAs in cells of the ovarian cancer line A2780 and in their cisplatin-resistant counterparts, A2780cis. Using these paired cell lines, we found that cisplatin-resistance was associated with augmented migration and invasion. Expression of miR-513a-3p was significantly diminished in A2780cis cells compared to cells of the parent A2780 line. Overexpression of miR-513a-3p resulted in inhibited cell migration and invasion, and additional results suggested that miR-513a-3p inhibited EMT through suppressing homeobox (HOX) B7. Cisplatin-resistance was associated with elevated levels of miR-513a-3p. This study suggests that miR-513a-3p and HOXB7 may play important roles in the pathogenesis and ultimately prognosis of ovarian cancer.

Materials and Methods

Cell Culture

Ovarian cancer cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Cat: A1049101, Rockville, MD, USA) containing 10% fetal bovine serum (Gibco, Cat: 10099, Gaithersburg, MD, USA) and 1% penicillin-streptomycin solution (Gibco, Cat: 15070063, Gaithersburg, MD, USA) at 37°C with 5% CO_2. Cells were sub-cultured in a ratio of 1:3 to 1:4.

Cell Transfection

To augment or reduce endogenous expression of target genes, A2780 cells or A2780cis cells were transfected with specifically designed siRNA. 10^5 cells were seeded into 6-well plates and transfected after confluence reached 70%-80%. Cell transfection experiments were conducted using the lipofectamine RNAiMAX transfection reagent (Invitrogen, Cat: 13778150, Carlsbad, CA, USA). After transfection, qRT-PCR assay was performed to verify the efficiency. The primer for miR-513a-3p mimics was 5'-UAAAUUUCACCUUUCUGAGAAGG-3' (sense), and 5'-UUCUCAGAAAGGUGAAAUUUAUU-3' (anti-sense); the primer for negative control mimics (NC mimics) was 5'-UUCUCCGAACGUGUCACGUTT-3' (sense), and 5'-ACGUGACACGUUCGGAGAATT-3' (anti-sense).

MTT Assay

To determine the sensitivity to cisplatin, 10^4 suspended cells in each group were plated into 96-well plates in triplicate. After cells had attached, the medium was changed into 200 µl fresh medium with 10% FBS and a 20 µl MTT (Beyotime, Cat: ST316, Shanghai, China) solution per well. After incubation for 4 hours, the medium was changed into 200 µl DMSO (Beyotime, Cat: ST038, Shanghai, China). After shaking for 10 mins, the OD values were measured by Thermo MK3 (Thermo Fisher Scientific, Waltham, MA, USA) at 490 nm. Cisplatin was diluted into DMSO; the doses of cisplatin ranged 0 µm to 50 µm.

qRT-PCR Assay

Cells were washed twice in phosphate-buffered saline (PBS; Gibco, Cat: 10010023, Rockville, MD, USA) and collected by centrifugation. Total RNA was extracted according to the instructions of the TRIzol kit (Qiagen, Cat: 80244, Waltham, MA USA). 1 µg RNA was reversed transcribed into cDNA according to the instructions of the PrimeScipt RT reagent kit (TaKaRa, Cat: RR047A, Otsu, Shiga, Japan). cDNA was used for qRT-PCR according to the instruction for detection with SYBR Green Mix (TaKaRa, Cat: DRR096A, Otsu, Shiga, Japan). The sequences of primers used in the study are listed below. GAPDH was used as a normalization control. Three independent experiments were performed. The primer for GAPDH was 5'-TCAGTGGTGGACCTGACCTG-3' (sense), and 5'-TGCTGTAGCCAAATTCGTTG-3' (anti-sense); the primer for E-cadherin was 5'-AAAGGCCCATTTCCTAAAAACCT-3' (sense), and 5'-TGCGTTCTCTATCCAGAGGCT-3' (anti-sense); the primer for N-cadherin was 5'-TCAGGCTGTGGACATAGAAACC-3' (sense), and 5'-GCTGTAAACGACTCTGGCACT-3' (anti-sense); the primer for vimentin was 5'-GACGCCATCAACACCGAGTT-3' (sense), and 5'-CTTTGTCGTTGGTTAGCTGGT-3' (anti-sense); the primer for HOXB7 was 5'-TTCCCAGAACAAACTTCTTGTGC-3' (sense), and 5'-GCATGTTGAAGGAACTCG-

GCT-3' (anti-sense); the primer for miR-513a-3p was 5'-TAAATTTCACCTTTCTGAGAAGG-3' (sense), and 5'-GCGAGCACAGAATTAATAC-GAC-3' (anti-sense); the primer for U6 was 5'-CGCTTCGGCAGCACATATACTA-3' (sense), and 5'-CGCTTCACGAATTTGCGTGTCA-3' (anti-sense).

Western Blot

Cells were washed twice with PBS and collected. Total protein was extracted using a Protein Extraction Kit (Applygen, Cat: P1250, Beijing, China). The concentration of these protein samples was measured with a bicinchoninic acid assay (BCA) protein assay kit (Thermo Fisher Scientific, Cat: NCI3327CH, Waltham, MA, USA). Approximately 30 to 50 μg protein samples were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Scientific, Cat: 88520, Waltham, MA, USA). The membranes were blocked in 5% non-fat milk at room temperature for 1-2 h and incubated with the primary antibodies against E-cadherin (Cell signaling Technology, Cat: 14472S, Waltham, MA, USA), N-cadherin (Cell signaling Technology, Cat: 4061S, Waltham, MA, USA), vimentin (Cell Signaling Technology, Cat: 5741S, Waltham, MA, USA) or HOXB7 (Santa Cruz Biotechnology, Cat: sc-81292, Santa Cruz, CA, USA) at 4°C overnight. The membranes were washed and then probed with corresponding secondary antibodies at room temperature for 1-2 hours. The membranes were imaged using an enhanced chemiluminescence (ECL) system (Thermo Fisher Scientific, Cat: 32106, Waltham, MA, USA) and analyzed by Bio-Rad Image Lab Software 5.2. Membranes were probed with anti-GAPDH (Cell Signaling Technology, Cat: 5174S, Waltham, MA, USA) to determine equivalent loading. Three independent experiments were performed.

Transwell Assay

Transwell assays were conducted by using the chambers with 8 mm polycarbonate membranes (Costar, Cat: 3422, Corning, Corning, USA) that were pre-coated with Matrigel (BD, Cat 354234, Franklin Lakes, NJ, USA). Cells in each group were collected and washed twice with PBS. The suspended cells were adjusted to a concentration of 10^6 cells/ml with serum-free medium and 100 μl aliquots of suspended cells were added to the upper chamber and incubated at 37°C with 5% CO_2. Six-hundred μl medium containing 10% fetal bovine serum was added to the bottom chamber. After 24 hours, the upper chamber membranes were washed twice with PBS, fixed with 4% paraformaldehyde (Beyotime, Cat: P0099, Beijing, China) for 15 mins and stained with 0.5% Crystal Violet (Beyotime, Cat: C0121, Beijing, China) for 15 mins at 37°C. The Crystal Violet-stained membranes were imaged under a 20 × microscope (Leica, Allendale, NJ, USA) and cells were counted by Image J. Three independent experiments were performed.

Wound-Healing Assay

Cells were collected and washed twice in PBS. The concentration of suspended cells in each group was adjusted to 10^6 cells/ml with RPMI-1640 medium containing 10% fetal bovine serum and seeded into 6-well plates. After cells attached, the monolayers were manually scratched with 10 μl sterile pipette tips and washed twice with PBS; the scratched areas were imaged; the time point was recorded as zero hours. After 48 hours incubation, cells monolayers were washed twice with PBS and the same healing areas were imaged. The experimental conditions in the experimental group were the same as those in the control group. Three experiments were repeated independently.

Colony Formation Assay

The cells were suspended and seeded into 6-well plates at a density of 10^3 per well. After 2 weeks, these plates were washed twice with PBS, fixed with 4% paraformaldehyde for 15 min and stained with 0.5 % Crystal Violet for 15 mins at 37°C. The colonies that included 50 cells or more were scored. The experiments were performed in triplicate and repeated independently.

Dual-Luciferase Reporter Assay

To explore the association between HOXB7 and miR-513a-3p, we focused on the complementary binding sites in HOXB7 (shown in Figure 4A). We constructed the Luciferase reporter plasmids that include HOXB7 and miR-513a-3p. The Dual-Luciferase reporter assay was performed according to the manufacturer's protocols using the Dual-Luciferase Reporter Assay Kit (Promega, Cat: E1910, Madison, WI, USA). Cells were transfected with Luciferase reporter and control plasmids. After 24 hours, the transfected cells were collected, and Luciferase activity was analyzed. The Dual-Luciferase reporter assays were performed in triplicate and repeated.

In Vivo Tumorigenesis

The target cells in log phase with high-expression of miR-513a-3p or miR-513a-3p/HOXB7 were washed twice with ice-cold PBS and suspended at 10^7 cells per ml with PBS. Two hundred µl of suspended cells were injected into the left and right hind flanks of six- to eight-week-old male nude mice by subcutaneous injection. Tumors were measured over a period of 3 days before the mice were killed. After one month, mice were killed, and tumor samples were analyzed by qRT-PCR and Western blot experiments. Tumor volume (mm^3) is calculated by the formula $V = L \times B^2 / 2$. V represents volume, L represents length, and B represents breadth of tumor measured in mm. All animal care and handling were in compliance with Institutional Animal Care and Use Committee.

Statistical Analysis

In the study, three experiments were performed in triplicate with three full replicates. Unpaired two-tailed Student's *t*-tests were performed using GraphPad Prism software. Statistics were significant when * refers to p-values < 0.05, *** refers to p-values < 0.01, and *** refers to p-values < 0.001.

Results

Cisplatin-Resistance Results in EMT and Reduced Sensitivity in Ovarian Cancer Cells Culture

To evaluate cisplatin sensitivity of ovarian cancer cells (cells of the A2780 line) and cisplatin-resistant cells (cells of the A2780cis line), we determined the half maximal inhibitory concentrations (IC50) values by MTT assay; generally, a lower IC50 value indicates a higher resistance to cisplatin. As shown in Figure 1A, the IC50 value for cisplatin was roughly tripled-increased in A2780cis cells compared to that determined for the parent A2780 cells. These results indicate that, as anticipated the sensitivity to cisplatin was dramatically reduced in the A2780cis cells. We then evaluated the capacity of the cisplatin-resistant cells to undergo migration, invasion, and colony formation. In the colony formation assay, we detected a six-fold increase in the number of individual colonies among the A2780cis cells than in the cells from the parent A2780 line (Figure 1B). Likewise, in the transwell assay, we identified 4-times more invading A2780cis cells (Figure 1C), and the wound-healing efficiency was increased 4-fold compared to the parent A2780 cells (Figure 1D). We conclude from these findings that that insensitivity to cisplatin is associated with increased capacity for cell migration, invasion, and colony formation. Western blot and qRT-PCR analysis demonstrated decreased levels of E-cadherin and increased levels of N-cadherin and vimentin in the A2780cis cells (Figures 1E and 1F); these results suggest that relative insensitivity to cisplatin may contribute to EMT in ovarian cancer cells.

Decreased Expression of MiR-513a-3p Correlated with the Relapse and Prognosis of Ovarian Cancer

We characterized differential expression of miRNAs expressed using a transcriptomics approach. The results of this study revealed relative down-regulation of the miR-513a-3p in the A2780cis cells (Figures 2A and 2B). Results from qRT-PCR verified this observation, which was also observed in cells from tumor tissues from ovarian cancer patients who had relapsed after chemotherapy with cisplatin (Figure 2C). Analysis of survival curves revealed that lower levels of miR-513a-3p in ovarian cancer tissue were associated with significantly reduced survival rates (Figure 2D). These results suggested that we might explore the use of miR-513a-3p as a biomarker for prognosis and recurrence of ovarian cancer, particularly in those patients who underwent treatment with cisplatin.

MiR-513a-3p Limited EMT and Promoted Cisplatin Sensitivity of Cisplatin-Resistant Tumors from Ovarian Cancer Patients

To determine the influence of heterologous production of miR-513a-3p on EMT in the cisplatin-resistant cell line, we utilized miR-513a-3p mimetics which resulted in a significant increase in the endogenous expression of miR-513a-3p in A2780cis cells (Figure 3A). Interestingly, overexpression of miR-513a-3p resulted in a decreased IC50 for cisplatin in A2780cis cells (Figure 3B); these results suggested that miR-513a-3p may directly improve cisplatin sensitivity. Moreover, the results of colony formation assay revealed that overexpression of miR-513a-3p limited colony formation (Figure 3C). Transwell and wound-healing assays revealed overexpression of miR-513a-3p in A2780cis cells also resulted in decreased efficiency of cell invasion and migration (Figures 3D and 3E) and Western blot and qRT-PCR analysis of these cells revealed increased expression of E-cadherin and diminished expression of both

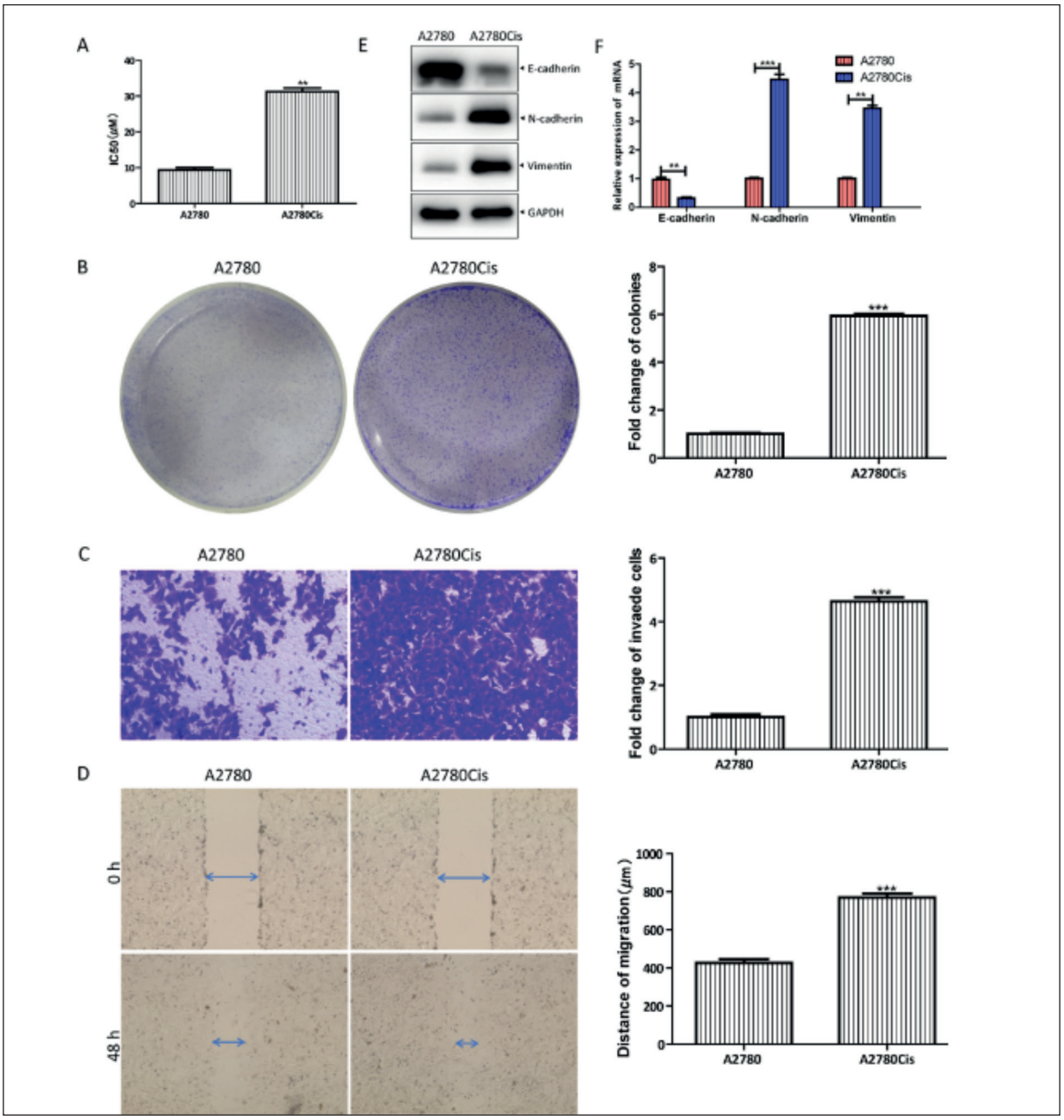

Figure 1. Decreased-sensitivity to cisplatin was associated with EMT in ovarian cancer cells. **A,** A2780 and A2780cis cells were plated at 10^4 cells per well into 96-well plates. The half maximal inhibitory concentration (IC50) values were measured with the MTT assay. **B,** Colonies were counted to evaluate the growth and sensitivity of ovarian cancer cells. **C,** Transwell assays were performed to detect the movement of ovarian cancer cells (magnification × 20). **D,** The wound-healing assay was performed to evaluate capacity for migration (magnification × 4). **E, F,** Western blot analysis and qRT-PCR experiments were performed to evaluate transcripts and proteins associated with EMT.

N-cadherin and vimentin, suggesting inhibition of EMT (Figure 3F and 3G). Taken together, these results suggest that overexpression of miR-513a-3p may enhance the sensitivity to cisplatin and suppress EMT in cisplatin-resistant ovarian cancer cells.

MiR-513a-3p Mediated Expression of HOXB7

Several reports have indicated that miRNAs can bind to complementary sites within the 3-untranslated regions (UTRs) of specific target genes[11]. We searched several online

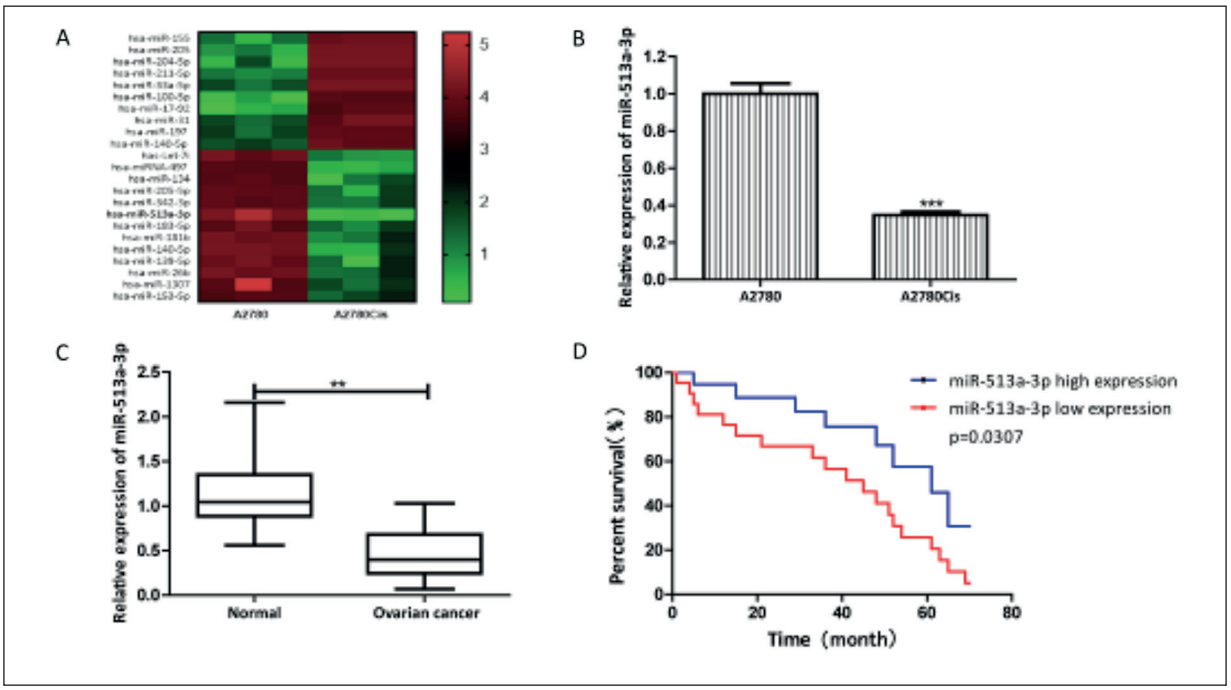

Figure 2. MiR-513a-3p was down-regulated in A2780cis cells and was associated with prognosis of ovarian cancer patients. **A,** Differential expression of miRNAs in A2780 and A2780cis cells. **B,** Expression of miR-513a-3p was down-regulated in A2780cis cells compared to A2780 cells as measured by qRT-PCR. **C,** Expression level of miR-513a-3p in tumor tissues from ovarian cancer patients who relapsed after cisplatin chemotherapy. **D,** Kaplan-Meier analysis was performed to determine the relationship between miR-513a-3p expression and survival rates among patients who relapsed after cisplatin treatment for ovarian cancer.

databases including starBase v2.0 and TargetScan, and identified HOXB7 as a potential target of miR-513a-3p. We also predicted candidate binding sites for miR-513a-3p within the 3-UTR of HOXB7 (Figure 4A). We constructed Dual-Luciferase reporter plasmids to verify the influence of miR-513a-3p on HOXB7 transcription. Dual-Luciferase reporter experiment showed overexpression of miR-513a-3p resulted in a profound decrease in Luciferase activity from the

HOXB7 reporter construct (Figure 4B); the expression of native HOXB7 also responded to miR-513a-3p overexpression (Figure 4C) and introduction of an miR-513a-3p inhibitor resulted in an increase in HOXB7 expression (Figure 4D). Furthermore, expression of HOXB7 was reduced in tumor tissue from ovarian cancer patients who relapsed after cisplatin chemotherapy (Figures 4E and 4F) and survival rates were inversely associated with the levels of HOXB7 detected (Figure 4G). These results demonstrated that miR-513a-3p may be a direct suppressor of HOXB7 expression.

MiR-513a-3p Inhibited EMT Promoted by HOXB7 and Improved Cisplatin Sensitivity

HOXB7 promotes the development of ovarian cancer and gastric cancer by regulating the EMT[12,13]. Stable knockdown of HOXB7 in A2780cis cells resulted in an increase in cisplatin sensitivity (Figure 5A). Similarly, colony formation, cell migration, and invasion were all significantly decreased in response to the stable HOXB7 knockdown in A2780cis cells (Figures 5B-D). Furthermore, Western blot and qRT-PCR analysis revealed increased protein and mRNA encoding E-cadherin and decreased levels of N-cadherin and vimentin in these cells (Figures 5E and 5F). These results revealed that HOXB7 might be promoting the metastatic potential of ovarian cancer. Specifically, our results indicate that a miR-513a-3p inhibitor could improve the capacity for cell migration and invasion and modify the sensitivity to cisplatin in the HOXB7-knockdown A2780cis cells (Figures 5A-F), suggesting that miR-513a-3p inhibitor can reverse the effects of HOXB7 in the A2780cis cells (Figure 5). Taken together, our results suggest that miR-513a-3p may be a primary

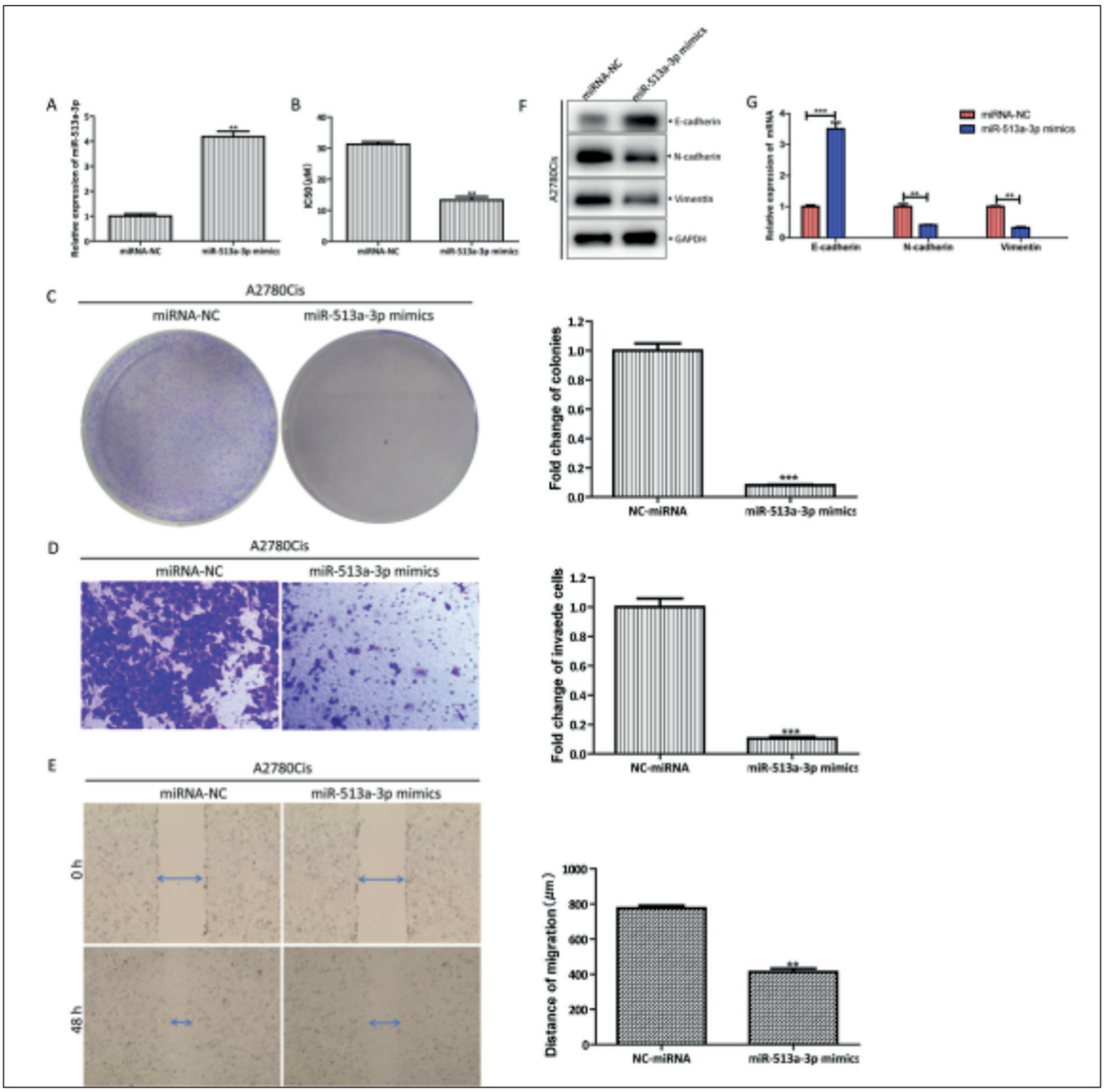

Figure 3. Overexpression of miR-513a-3p limited EMT and increased cisplatin sensitivity in cisplatin-resistant ovarian cancer cells. **A,** Expression of miR-513a-3p in cells transfected with miRNA-NC or miR-513a-3p mimetic evaluated by qRT-PCR. **B,** Overexpression of miR-513a-3p enhanced the sensitivity to cisplatin in A2780cis cells. **C,** Overexpression of miR-513a-3p limited colony formation. **D,** Overexpression of miR-513a-3p suppressed migration and invasion of A2780cis cells (magnification × 20) in transwell assays. **E,** Overexpression of miR-513a-3p weakened the ability of A2780cis cells to recover from wounds (magnification × 4). **F-G,** Detection of E-cadherin, N-cadherin, and vimentin by qRT-PCR and Western blot in response to overexpression of miR-513a-3p.

mediator of HOXB7-induced EMT and regulated cisplatin sensitivity.

MiR-513a-3p Inhibited Growth of Xenograft Ovarian Tumors Via Interactions with HOXB7

To evaluate the impact of miR-513a-3p on tumor growth *in vivo*, A2780cis cells transfected with miR-513a-3p-agonist were injected into nude mice. Results showed that growth of xenograft tumor was slower in the miR-513a-3p-agonist group compared to NC-agonist group (Control group), including the decreased volume, reduced weight, and poor survival of tumor samples (Figures 6A-E). The growth of xenograft tumor was more rapid in the miR-513a-3p-agonist + HOXB7 group

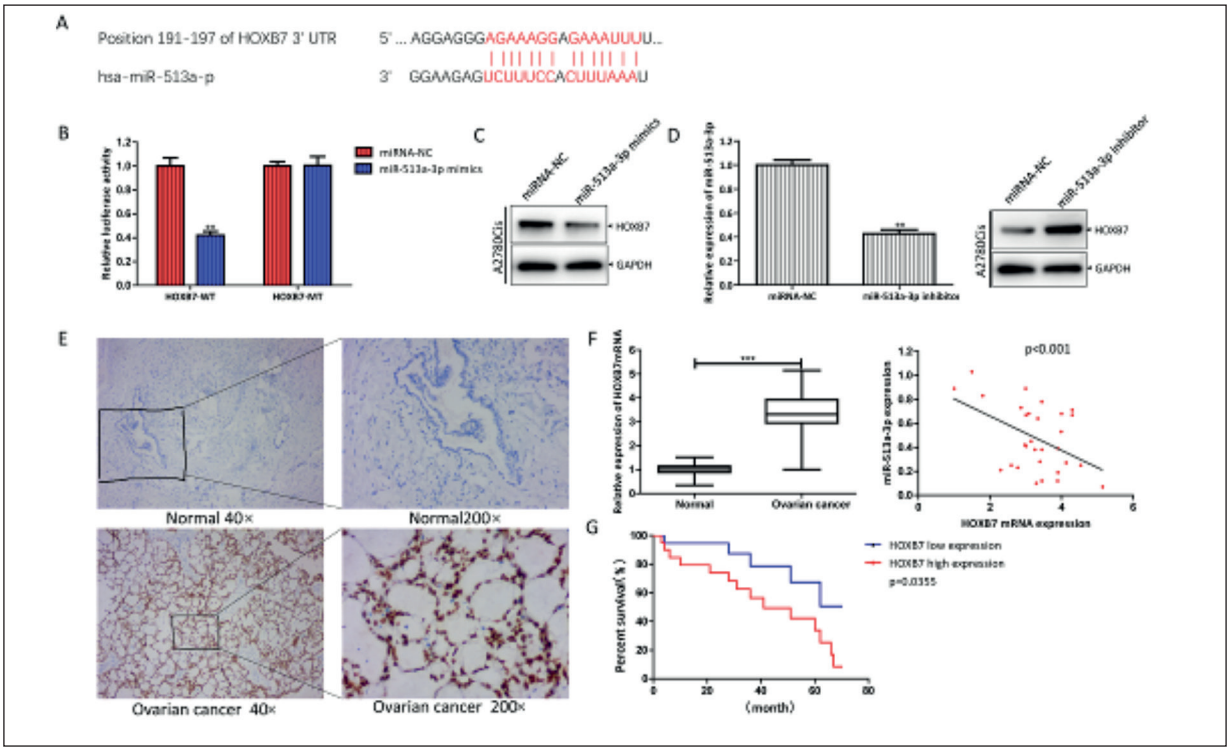

Figure 4. MiR-513a-3p inhibited the expression of HOXB7. **A,** The predicted binding sites for miR-513a-3p on the 3-UTR of HOXB7 were revealed by searches in the starBase v2.0 and TargetScan databases. **B,** Cells were co-transfected with Dual-Luciferase reporter Luciferase activity was measured according to the instrument of Dual-Luciferase reporter assay. **C,** Western blots probed with anti-N-cadherin, anti-E-cadherin, and anti-vimentin. **D,** qRT-PCR and Western blot assays were performed to detect the expression levels of miR-513a-3p and HOXB7 in cells provided with an miR-513a-3p inhibitor. **E,** Immunohistochemical detection of HOXB7 in tumor tissue from ovarian cancer patients who relapsed after cisplatin chemotherapy (magnification × 200). **F,** Expression of HOXB7 was detected by qRT-PCR and correlation with HOXB7 and miR-513a-3p expression tumor tissue from ovarian cancer patients. **G,** Kaplan-Meier analysis of the survival rates of ovarian cancer patients who relapsed after cisplatin chemotherapy.

with increased expression of HOXB7 compared to those in the miR-513a-3p-agonist group; these results suggested that HOXB7 counteracts the impact of miR-513a-3p in this setting (Figures 6A-E). In addition, the relative protein and expression of transcripts encoding proteins related to EMT were measured by Western blot and qRT-PCR (Figure 6F, 6G). Our results demonstrated that miR-513a-3p and HOXB7 both had an impact on EMT in ovarian cancer and that HOXB7 rescued the inhibitory effects on miR-513a-3p overexpression. Overall, our results suggest that miR-513a-3p may play a vital role on the development of ovarian cancer via its actions on HOXB7-induced EMT.

Discussion

Ovarian cancer is one of the most common malignancies among women worldwide and has a very high mortality[2,14]. Previous studies[1,2] have reported that nearly 80% of ovarian cancer patients have been in advanced stage when diagnosed notably due to the lack of early and effective detection methods. Surgical management followed by platinum-based chemotherapy is currently the standard of care for ovarian cancer patients. However, drug resistance is a non-negligible concern in clinical treatment. Drug resistance can have a significant impact on the ultimate efficacy of chemotherapy treatment and may promote metastasis and release, and is associated with an overall poor prognosis[3,4]. While cisplatin has been used widely for the treatment of ovarian cancer, cisplatin-resistance can occur[5]. EMT is one of the most important factors contributing to drug resistance. Drug resistance has been associated with activation of EMT, and can include increased migration, invasion, and reduced apoptosis in breast, cervical, and ovarian cancer cells[15-17]. TGF-β may

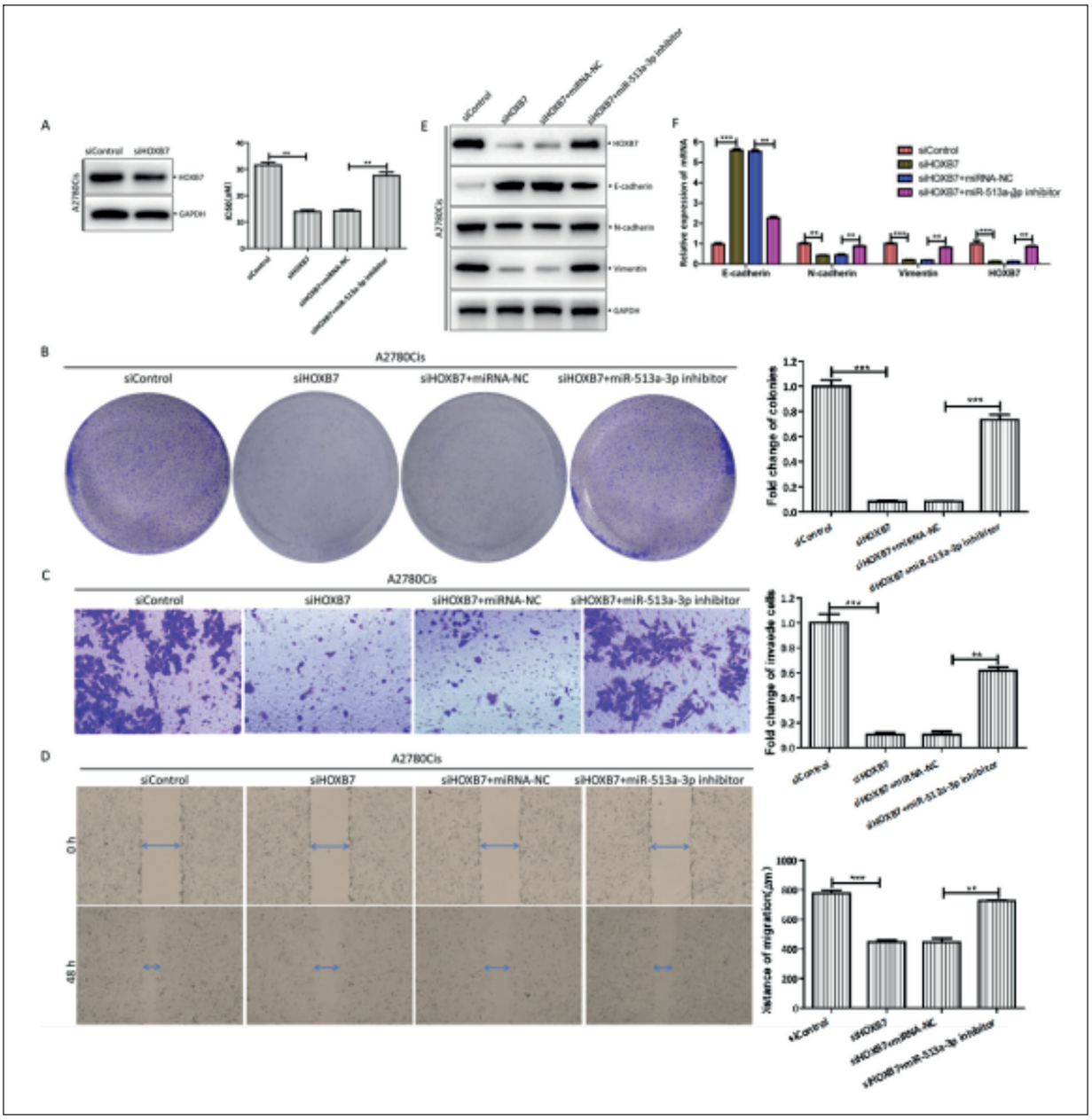

Figure 5. MiR-513a-3p inhibited EMT promoted *via* HOXB7 and improved cisplatin sensitivity. **A,** qRT-PCR assay was performed to detect the efficiency of the knockdown treatment in A2780cis cells and the MTT assay evaluated cell viability and sensitivity to cisplatin in the stable HOXB7 knockdown cells. **B,** Colony formation assay. **C-D,** Transwell (magnification × 20) and wound-healing assays (magnification × 4) were conducted to evaluate the capacity for cell migration and invasion in the various cell lines. **E-F,** Expression of protein and mRNA were evaluated by qRT-PCR and Western blot.

be a major factor serving to promote metastasis and resistance to chemotherapy[18]. Likewise, reduced expression of apoptosis-stimulating protein of p53 (ASPP) 2 promotes EMT, increased 5-FU resistance, and anti-apoptosis in renal carcinoma cells[19]. As such, chemotherapy-induced EMT may be related to drug resistance in cancer. Clarification of the mechanisms contributing to EMT will be crucial and primary for the treatment and prognosis of ovarian cancer patients with drug resistance.

MicroRNAs are short non-coding RNAs transcribed by RNA polymerase II and are involved in various cancers. MicroRNAs are crucial parts of capped and polyadenylated primary transcripts and can be either protein-coding or non-coding.

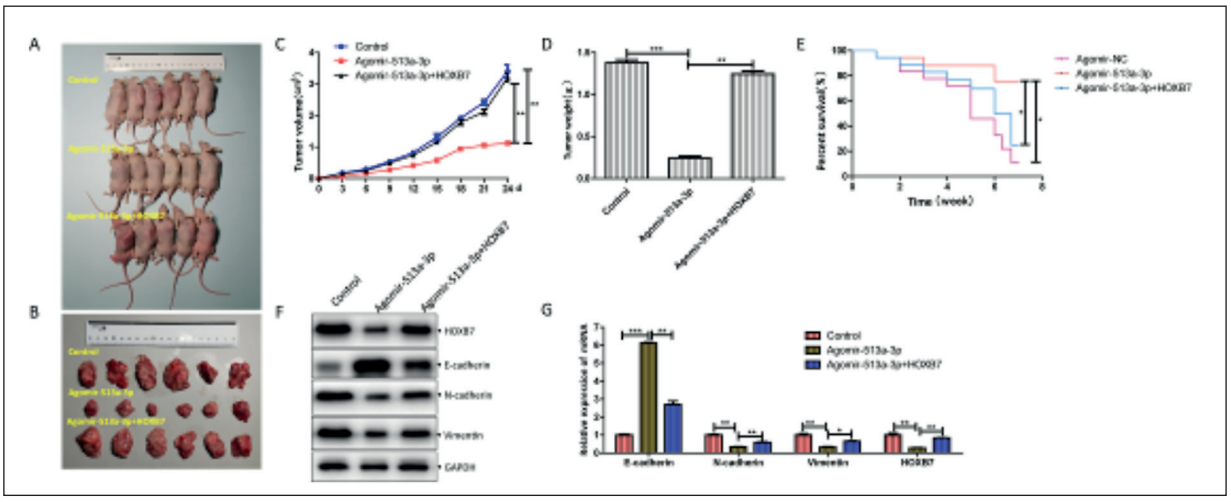

Figure 6. MiR-513a-39 inhibited growth and survival of ovarian cancer *via* EMT. **A-B,** In *vivo* tumorigenesis experiment was conducted in nude mice to evaluate the effect of miR-513a-3p. At 14-days after injection, the size of tumor samples was measured in each group. **C-D,** Volume and weight of tumor samples were measured in each group. **E,** Survival rate was analyzed. **F-G,** Western blot and qRT-PCR assays were performed to detect the markers involved in EMT.

MicroRNAs play pivotal roles in the development and metastasis of tumors and other diseases. Their actions can have a critical impact on cell proliferation, migration, apoptosis, metabolism, and other processes[7,20,21]. Notably, reduced expression of miR-142-3p inhibits proliferation, invasion, and apoptosis of renal carcinoma cells and pancreatic cancer cells[22,23]. MiR-15/16 mediates differentiation of amniotic epithelial cells through mediating the crosslink between the MAPK and Wnt/β-catenin pathways[14] and MiR-15 inhibits the progression of glioblastoma *via* its interactions with insulin-like growth factor receptor 1[20]. Chen et al[21] revealed that aberrant-expression of miR-513a-3p has a direct impact on the development and metastasis of gastric cancer, as increased expression inhibits cell proliferation, migration and promotes apoptosis by targeting high mobility group-box 3 protein. Likewise, miR-513a-3p promotes dichlorvos-induced apoptosis *via* its interactions with B-cell lymphoma (Bcl-2) in HK-2 cells[24]. MiR-513a-3p regulates the expression of luteinizing hormone/chorionic gonadotropin receptor genes in granulosa cells[25]. Numerous miRNAs participate in chemotherapy resistance and EMT of cancer cells by regulating expression of target genes[6,7]. MiR-200b, miR-15b, and miR-3656 play crucial roles in mediating chemotherapy-induced EMT and drug resistance[8-10]. In this study, miR-513a-3p expression was significantly decreased in cisplatin-resistant ovarian cancer cells, while overexpression of miR-513a-3p promoted cisplatin sensitivity and inhibited cell migration and invasion, that it may have avital impact on the metastasis and cisplatin-resistance of ovarian cancer. Furthermore, we predicted HOXB7 was a target gene of miR-513a-3p by utilizing starBase v2.0 and TargetScan.

HOXB7 is a member of the Antennapedia (Antp) homeobox family; its transcript encodes a protein with a homeobox DNA-binding domain. HOXB7 can be detected in numerous cells and tissues[26-28] and has been associated with both melanoma and ovarian carcinoma. As reported, overexpression of HOXB7 induces cellular proliferation and poor prognosis in oral cancer[29] while diminished expression inhibits the tumorigenesis and progression of osteosarcoma[30]. MicroRNAs miR-376c-3p and miR-196b-5p regulate metastasis and cellular apoptosis by promoting EMT *via* interactions with HOXB7[31,32]. However, the relationship between miR-513a-3p and HOXB7 had not been explored specifically in ovarian cancer. As shown in this manuscript, the biological impact of miR-513a-3p in ovarian cancer cells was reversed by HOXB7, and we present evidence suggesting that miR-513a-3p inhibits the HOXB7-mediated EMT signaling pathway.

Conclusions

The expression of miR-513a-3p was decreased in ovarian cancer cells and in tumors from pa-

tients who had relapsed after cisplatin chemotherapy; these findings were accompanied by increased expression of HOXB7. While cisplatin-resistance was associated with cell invasion, migration, and colony formation, down-regulation of miR-513a-3p promotes cisplatin sensitivity and is associated with improved prognosis. Taken together, our study analyzes the biological mechanism of HOXB7 through miRNA. These results indicate that miR-513a-3p may have a profound effect on metastatic potential *via* HOXB7 as well as cisplatin sensitivity in ovarian cancer. The discovery might provide available information on potential new targets for the diagnosis, prognosis, and treatment for ovarian cancer patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Yang Chen and Yun Zhao conceived and designed the experiments; Yong Chen and Xiuhong Zhao performed the experiments; Dongdong Zhang performed the data analysis; Yong Chen, Xiuhong Zhao and Yun Zhao contributed to the manuscript. All authors have read and agreed to the final submitted version of the manuscript.

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